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KARYOLOGICAL STUDIES OF JAPANESE ANTS II. SPECIES DIFFERENTIATION IN *APHAENOGASTER*; WITH SPECIAL REGARD TO THEIR MORPHOLOGY, DISTRIBUTION AND CHROMOSOMES[1]

By Hirotami T. Imai[2]

The species of *Aphaenogaster* are among the most familiar ants in Japan, and three species, *A. osimensis*, *A. famelica*, and *A. smythiesi* have been recognized (Yano, 1910; Teranishi, 1915, 1940; Wheeler, 1928; Morishita, 1945; Okamoto, 1954).

Species-level studies, however, have seldom been made on the *Aphaenogaster* group, except some identification of species on the basis of the external morphology of the worker caste (Emery, 1908; Smith, 1961). As already discussed in part I of this series (Imai, 1969), the combination of geographical distribution and karyotype analysis gives us excellent information contributing to the analysis of species differentiation in ants. In this connection, the three Japanese species of *Aphaenogaster* are especially suitable materials, because they have distinct but interrelated distribution patterns and karyotypes.

In this paper, the detailed species differentiation of *Aphaenogaster* will be analysed by distribution and karyotype studies, and after due consideration, the phylogenetic usefulness of the orthodox external morphology will be examined.

Materials and Methods

The three species used for morphological comparison were collected during July to August in 1966; *A. osimensis* from Manazuru peninsular in Kanagawa Prefecture, *A. famelica* from Mt. Ohoyama in Kanagawa Pref., *A. smythiesi* from Asamushi in Aomori Pref. and Mt. Takao near Tokyo.

Maturated winged males and queens, and workers were fixed with modified

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Carnoy's solution (methyl alcohol 1 part and glacial acetic acid 1 part) for five minutes and pasted on a graduated transparent tape. Drawings were made by tracing figures reflected on section paper. Analysed characters were selected with those characters; 1) remarkably different among three species, and 2) common to two species but different in one species. The characters were analysed by using all castes, queen, worker, and male.

The great part of the specimens used for ecological distribution were from the collections housed in the Entomological Laboratory of Kyushu University. A few collections by Drs. M. Morishita and Y. Murakami were drawn upon, and further specimens were by Dr. M. Kubota, Dr. M. Kondo, Dr. K. Hayashida, Mr. H. Okamoto, and Dr. J. Hasegawa. Living materials from Sapporo in Hokkaido were donated by Dr. K. Hayashida. The other field collections were made by the present author. The main check points to distingnish each species were based on the rugosity pattern of head and alitrunk of worker and queen. Especially in the workers the rugosity of pronotum (prothorax) and the degree of prominence of mesonotum (mesothorax) were found to be the most reliable characters for the morphological identification of the three species.

For the chromosome studies, males, queens, and workers were collected from field and an artificial colony. Mostly, males developed parthenogenetically from worker eggs were used; these were determined to possess the same chromosome number as normal field males (Imai, 1966). The haploid chromosome numbers were observed in the brain cells and spermatocyte cells of males and the diploid number was determined from the brain cells of queens and workers, and also on the oogonial cells of queens. For the chromosome preparation, the aceto-orcein squash method was applied. Organs of suitable stages ("transparent" stage of prepupae, "slight rouge" eye stage of male pupae, and "scarlet" eye stage of queen pupae) was dissected out in Carlson's solution (Carlson, 1946) and kept for ten minutes in hypotonic solution (0.45% sodium citrate) at room temperature. After the hypotonic solution was removed the tissues were stained by 1% aceto-orcein (dissolved in 50% glacial acetic acid) and then squashed. The details of this method have been described in previous papers (Imai, 1966; part I of this series, 1969).

Observations

1. *Comparison of morphology*

The morphological characters observed in three species are summarized in the drawings in Plates 1-2. The correlations of observed characters in these species can be classified in three categories and are symbolized as follows:

1) Transient change in some character from one species to another is symbolized as →.
2) Obvious resemblance in some character of one species with another is symbolized as ∞.
3) Apparent difference in some character between two species is symbolized as //.

The five observed interspecific relations can be symbolized as 1) o→f→s, 2) o∞

Table 1. Phylogenetic relationships among species of the three Japanese *Aphaenogaster* based on the comparison of external morphologies.

Morphological characters		Female		Male
		Worker	Queen	
Shape	Head	o∞f // s	o∞f∞s	o // f∞s
	Frontal area	o∞f // s	o∞f // s	o∞f∞s
	Frontal edge of clypeus	o∞f∞s	o∞f // s	o // f∞s
	Alitrunk	o→f→s	o∞f∞s	o // f∞s
	Petiole	o∞f∞s	o∞f∞s	o∞f∞s
	Gaster	o∞f∞s	o∞f∞s	o∞f∞s
	Wing venation	——	o→f	o→f→s
Rugosity	Head	o→f→s	o→f→s	o∞f∞s
	Alitrunk	o→f→s	o→f→s	o∞f∞s
	Petioles	o // f∞s	o∞f∞s	o∞f∞s
	Gaster	o∞f∞s	o∞s // f	o∞f∞s
Size	Body	o∞f // s	o∞f // s	o∞s // f
	Leg	o∞f // s	o∞f // s	o∞s // f

o : *osimensis*, f : *famelica*, s : *smythiesi*, ∞ : Similar character states, // : Remarkably different character states, → : Transient character states.

f∞s, 3) o∞f // s, 4) o // f∞s, 5) o∞s // f. (o : *osimensis*, f : *famelica*, s : *smythiesi*). These five cases are shown in Table 1, and the other types of theoretical possibilities were not observed.

The most remarkable difference is observed in the rugosity patterns which cover head and alitrunk of worker and queen castes. The rugosity is shown to be a character of type o→f→s. Rugae are weak in the worker of *osimensis* (Plate 1, figs. 7, 8), in which the head is impressed with fine striations only in the anterior half, with the posterior half being smooth and shining. Pro- and mesonotum are also smooth and shining in *osimensis*.

On the other hand, in the worker of *famelica* (Plate 1, figs. 9, 10) the striations of head are deeply engraved to make a rugoreticulum on the posterior part. The puncturation of pronotum is increased in *famelica*, and the pronotum is smooth only on the sides. The mesonotum is densely engraved by deep branched rugae in this species. This rugosity is developed remarkably in *smythiesi* (Plate 1, figs. 11, 12), in which the head is completely covered with densely reticulate rugae, and the pronotum is also fully punctate and opaque. The most remarkable character of *smythiesi* is a steep prominence of the anterior part of mesonotum which projects in an angle higher than the pronotum. The rugosity can be also observed in the queens (Plate 1, figs. 1-3). In general, the queen caste has a more developed rugosity than that of the workers.

In the male caste of these three species (Plate 1, figs. 4-6), the rugosity is obsolescent (Plate 2, figs. 12-20) but the de-sclerotization of wing venation (Plate 2, figs. 3-5) shows the same sequential tendency, o→f→s. The wing venation of queens (Plate 2, figs. 1, 2) was compared for *osimensis* and *famelica*. These were also observed to exhibit the same differences as those of the males, in the increased de-sclerotization in the venation of *famelica*.

Relation o∞f // s is observed in the following three characters: The number of prominent lines of the frontal area in both worker and queen, the shape of the frontal edge of the clypeus in queen (Plate 2, figs. 6-8), and length of the legs both worker and queen (Plate 1, figs. 1-3, 7, 9, 11). In *osimensis* and *famelica*, one prominent line runs through the frontal area of both worker and queen, but no line occurs in the worker and three in the queen of *smythiesi*. The same relation is also found in the length of the legs. The former two species have very long legs but the last species has very short legs in both worker and queen. The form of the legs in *smythiesi* seems to be similar to that of members of *Myrmica*.

Relation o // f∞s is observed on the shape of the epinotum in male (Plate 2, figs. 18-20): *osimensis* is completely different from the remaining two species, in having a shape of long box epinotum without any projection of epinotal spine and of metapleural gland. The same relation is observed on the puncturation of the petiole and postpetiole of workers, which are smooth and shining in *osimensis* but are densely punctate in the remaining two species.

A relation o∞s // f is shown by the puncturation of the gastric surface of the queen caste (Plate 1, figs. 1-3), which is smooth and shining in *osimensis* and *famelica* but (only the queen of *famelica*) is punctate shallowly over the whole surface of abdomen, although the workers of *famelica* have smooth abdomens. The same relation is observed with the body size and leg length of the male caste (Plate 1, figs. 4-6), in which the male of *famelica* are larger than the males of the other two species.

2. *Distribution patterns*

A. osimensis: This species is subtropical, being distributed only in southern Japan. The nests are mostly found in rocky places along the coast of the Pacific Oceans, where the climate is warm even in winter. The nests are made in slits of rocks. This type of nesting behavior, characteristically found in tropical, is primitive as compared with that of highly socialized temperate species. The northernmost spot along the coast of the Pacific Ocean where specimens were collected was Otsu, Ibaragi Pref.; and specimens from the southernmost part of Japan was collected from Amami-Oshima. On the other hand, not a single specimen has ever been collected from the coast of the Japan Sea and from the coastal regions of Sanriku and Hokkaido (Fig. 1). As far as observed, it may be said that this species occurs only up to the southern coast of Kanto District in Japan. This northern limit of distribution agrees well with the isothermal line of −3.5°C (mean minimum temperature of the year). This distribution pattern is characteristic of many tropical species as mentioned by Imai (1969).

A. famelica: The northernmost distribution was recorded at Mt. Daisetsu of

Hokkaido, and the southernmost sample was collected from Cape Sata of Kyushu. Many samples were collected from Kyushu, Shikoku, and Honshu (Fig. 1). The habitat of this species is somewhat different from that of *osimensis*. Most of the nests are found in pebbly ground, in some cases in stone hollow and slits in rocks where the air was somewhat moist. No distinct overlap of habitat has been observed between *osimensis* and *famelica*. The nesting behavior of *famelica* is exhibited commonly by other temperate ant species.

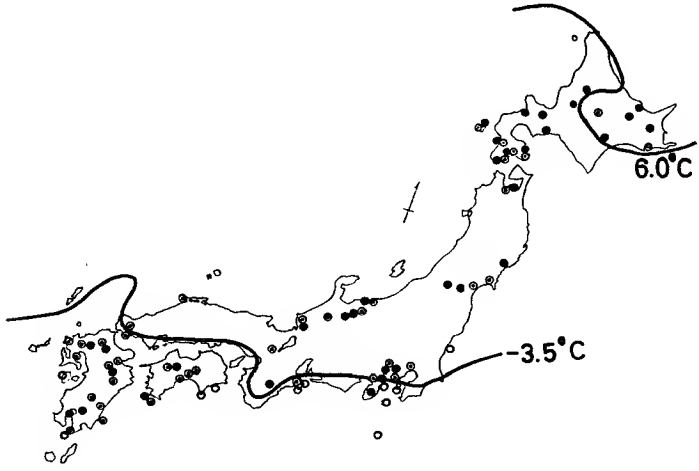


Fig. 1. Horizontal distribution of three Japanese *Aphaenogaster* ants.
○ *A. osimensis*, ⊙ *A. famelica*, ● *A. smythiesi*.
—3.5°C: The —3.5°C isothermal line of the mean minimum temperature of the year known as the northernmost boundary line of the tropical ants. 6.0°C: The 6.0°C isothermal line of the average temperature of the year known as the northernmost boundary line of the temperate ants. *osimensis* is found in rocky places along the coast of the Pacific Ocean more than south of —3.5°C line. *famelica* and *smythiesi* are found all over the Japan from Kyushu to middle of Hokkaido and show the same distribution pattern.

A. smythiesi: The present author was able to get this species at Abashiri as his most northern collections for the genus, and found it often in Honshu, Shikoku and Kyushu. The geographical distribution is pictured in Fig. 1. As far as the horizontal distribution is concerned, the pattern of this species resembles that of *famelica*. On the other hand, a remarkable difference between the two species was found in the vertical distribution (Fig. 2). For example, *smythiesi* is not found in the basal zone in southern Japan, namely, Kyushu, Shikoku, and the southern half of Honshu. In this southern part of the species' range, nests

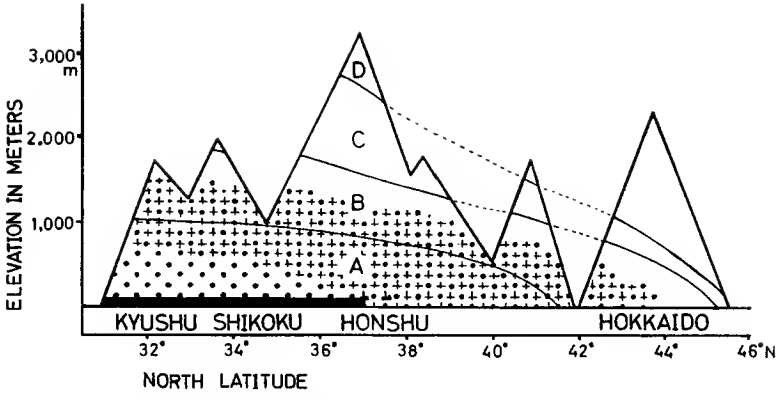


Fig. 2. Vertical distribution patterns of three *Aphaenogaster* ants compared with plant zones in Japan.
A. Basal zone. B. Mountain zone. C. Sub-Alpine zone. D. Alpine zone. ▬ *A. osimensis*, ·····*A. famelica*, +++ *A. smythiesi*.
The northern limit of the distribution of *osimens is* corresponds to the —3.5°C isothermal line at about 37°N, which agrees with the distribution of tropical type I species. Both *famelica* and *smythiesi* have the same upper limit of the vertical distribution agreed with the mountain zone but *smythiesi* does not appear at the level land in the southern part of —3.5°C isothermal line. The distribution pattern of *famelica* is characteristic in the temperate type I species, and that of *smythiesi* does temperate type II.

are found at 600 or 800 meters or higher.

The lowest level where nests are found approaches gradually to the basal zone in more northern part of Japan. It is about 300 meters high in Kyoto and at sea level farther to the north, in Oyashirazu, Niigata Pref., and in Asamushi, Aomori Pref.

Another difference exists in the nesting environment of *famelica* and *smythiesi*. Most of the nests of *smythiesi* seem to be made in field or copse. In spite of a considerable overlapping in horizontal and vertical distribution of both species, there is a segregation in habitat between them. One example was observed at Asamushi where the nests of *famelica* were found in slits of stone walls or under the stones of a pebbly road, but those of *smythiesi* were limited to the soil of copse about a few meters elevated from the coast level. As the latter type of habitat is commonly prefered by ant species centered in cold regions, it may be reasonable to consider tbat this species, *smythiesi*, is well adapted to a cold environment. It is, however, interesting to note that specimens which are identified morphologically as *smythiesi* are also found in Amami-Oshima, a locality within the margin of the subtropical zone.

3. *Karyotype analysis*

A. osimensis: A diploid chromosome number of $2n=32$ was observed in the 35 oogonical cells of queen pupae and also in 9 somatic cells of the brain of queen prepupae. Haploid sets of chromosomes of $n=16$ were counted in 16 first spermatocyte cells of male pupae. The chromosome constitution of the haploid set is shown by the following karyotype formula:

$n=1SM^{2SC}+1ST^{SC}+2M+2SM+10A=16$ (Plate 3, figs. 1, 2).

(Where, SM^{2SC}: Submetacentric cbromosome having one secondary constriction in both short and long arms; ST^{SC}: Subtelocentric chromosome having one secondary constriction in long arm; SM: Submetacentric chromosome; M: Metacentric chromosome; A: Acrocentric chromosome.)

A. famelica: A diploid chromosome number of $2n=34$ was observed in brain cells, 19 cells from queen and 15 cells from workers. Haploid set of chromosomes of $n=17$ was examined in 8 first spermatocytes of male pupae. The chromosome constitution of the haploid set is shown by the following formula:

$n=2T^{SC}+1ST^{SC}+2M+2SM+10A=17$ (Plate 3, figs. 3, 4).

(Where, T^{SC}: Telocentric chromosome having one secondary constriction in long arm.)

In contrast to the karyotype of *osimensis*, the SM^{2SC} chromosome does not occur in this species; while two telocentric chromosome, each of which has one secondary constriction, are found anew. The other components are the same as those of *osimensis*.

A. smythiesi: A diploid chromosome number of $2n=22$ was observed in 3 oogonial cells from queen pupae, and 19 somatic cells from brains of workers. Haploid sets of chromosome of $n=11$ were counted in 50 first spermatocytes of male pupae. The karyotype formula of the haploid set is as follows:

$n=10SM+1M$ (Plate 3, figs. 5, 6).

This formula is entirely different from those of *osimensis* and *famelica*. Namely, the largest SM chromosome which corresponds to SM^{2SC} of *osimensis* has no secondary constrictions; and no acrocentric chromosome is observed in this species, whereas ten acrocentric chromosomes are counted in other two species.

Discussion

From the morphological view point, excellent characters are available in the shape, rugosity pattern, and size of several body parts. The resemblance of each character was compared with the worker, queen, and male castes to be expressed by three symbols: 1) remarkably different character states (designated as $/\!/$), 2) similar character states (designated as ∞), and 3) transient character states (designated as $\rightarrow$). Based on these criteria five groups of interspecies correlation could be established: $o\rightarrow f\rightarrow s$, $o\infty f\infty s$, $o\infty f/\!/s$, $o/\!/f\infty s$, and $o\infty s/\!/f$ (where, o: *osimensis*, f: *famelica*, s: *smythiesi*) (Table 1). At first sight, no clean evolutional relation among these species seems possible, because it will be difficult to decide which groups of interspecies correlation represent the true phylogenetic correlation.

For phylogenetic analysis, it seems best to select those characters which show the least intraspecific variation. Considering the diphenism in the female sex, characters were selected in which the least difference occurs between the worker and queen. For this reason, those characters which exhibit some interspecific differences only in the queen, for example, the shape of frontal edge of clypeus (Plate 2, figs. 6-8) and the punctation of gaster (Plate 1, figs. 1-3), or only in the worker, for example, the profile of alitrunk (Plate 1, figs. 8-12), rugosity pattern of petioles, were eliminated. After this screening, two categories of interspecific correlation were remained.

The first category includes those characters showing either discontinuous or continuous variation between two of these species. These were expressed as o∞f // s and observed on the shape of the frontal area (Plate 2, figs. 6-8) and the length of legs and body size of female castes (Plate 1, figs. 1-3, 7, 9, 11). However, other types of correlations were found in the male caste (Plate 1, figs. 4-6; Plate 2, figs. 18-20), namely, o // f∞s, the shape of epinotum and o∞s // f, the size of body and legs. It is strange that female and male show a different pattern of interspecies correlation. One can see that with these observations it is still impossible to obtain a clear picture of phylogeny.

The second category includes stable characters showing continuous variations whose degree shows a sequential trend from one species to another (o→f→s). Rugosity of the head and thorax (Plate 1, figs. 1-3, 7-12; Plate 2, figs. 6-8, 9-11) is an example which appears constantly in all the individuals of female castes with different degrees of coarseness increasing sequentially from *osimensis* to *smythiesi*, with *famelica* as the intermediate. It is also a strange fact that in the male there are no differences observed in rugosity patterns (Plate 1, figs. 4-6; Plate 2, figs. 12-20). However, the same tendency was observed in the wing venation of male (Plate 2, figs. 3-5) and queen (Plate 2, figs. 1-2), in which the de-sclerotization of wing venation increase as a range of o→f→s. From these tendencies, a simple cladogram can be given as follows:

From the study of geographical distribution, *osimensis* is found at sea level in the southern part to the −3.5°C isothermal line (Fig. 1), the northern limit of distribution of tropical species. From its vertical distribution pattern, this species belongs to the class I have labeled tropical type I. The distribution of *famelica* extends horizontally from Kyushu to the middle of Hokkaido and vertically from the level land to the mountain zone (Fig. 2). This distribution pattern shows that *famelica* belongs to the class labeled temperate type I. In the case of *smythiesi*, the same pattern of horizontal distribution as that of *famelica* is observed, but the species is limited altitudinally to the mountain zone. This distribution pattern belongs to the class labeled temperate type II.

As already discussed in part I of this series (Imai, 1969), two major types of origins were showed in Japanese ants based on distribution patterns and karyotype analysis. One is the tropical origin, in which are included species of tropical type I and temperate type I. These species were postulated to have spread to Japan from tropical regions after the latest glacial age of this epoch, and are called "Neo" species. The remaining group of Japanese species is of temperate origin, in which are included species referred to as temperate type II. These species were considered to be presented in Japan before the latest glacial age of the Caenozoic, and are called "Relict" species.

As temperate type I is assumed to have differentiated from tropical type I, *famelica* seems to be closely related to *osimensis*. On the contrary, *smythiesi*, which belongs to temperate type II seems to be rather distant from other two species. Thus, by borrowing the symbols, "→" and "//", which were used for comparing morphological character, the phylogenetic relation could be expressed in the following ways: o→f // s.

Finally, the results of karyotype analysis of these three species are seen to have some phylogenetic utility. The karyotype formula of these three species are expressed as follows:

For *osimensis*, $n=1SM^{2SC}+1ST^{SC}+2M+2SM+10A=16$;
For *famelica*, $n=2T^{SC}+1ST^{SC}+2M+2SM+10A=17$;
For *smythiesi*, $n=1SM+1M+9SM=11$.

The karyotype evolution of these species can be explained by polyploidization and by centric dissociation which are assumed to be characteristic for the alteration of ant's karyotype. It is found that 16 haploid chromosomes of *osimensis* counted 8 pairs of morphologically "homologous" chromosomes as if 16 chromosomes are diploid (Plate 3, figs. 1, 2). The same condition is also found in the karyotype of *famelica* (Plate 3, figs. 3, 4). These evidences strongly suggest that the karyotype of *osimensis* and *famelica* duplicated from the common 8 haploid chromosomes, which could be assumed to be basic number of chromosomes in *Aphaenogaster*. As already mentioned in part I, the mode of basic number of Hymenoptera is $n=7$, 8, and 10 (White, 1954; Nogusa and Kato, 1962, 1963; Nogusa, 1965). Therefore the duplication theory of the *Aphaenogaster* karyotype is not incompatible with the general tendency of the karyotype evolution found in Hymenoptera.

The karyotype of *smythiesi* is much different from those of the other two species (Plate 3, figs. 5, 6). However, there are several common chromosomes, namely, one largest submetacentric, one middle sized submetacentric chromosomes. In order to identify the common ancestral karyotype of these three species, the following three assumptions have to be made. First, the middle sized acrocentric chromosomes of *osimensis* and *famelica* were induced from submetacentrics found in *smythiesi* by inversion. Second, the karyotypes of *osimensis* and *famelica* were brought about by a duplication from the basic karyotype of $n=8$. Third, partial polyploidization of submetacentric chromosomes should occur in the karyotype of *smythiesi*. On these assumptions, the common ancestral karyotype could be deduced as follows:

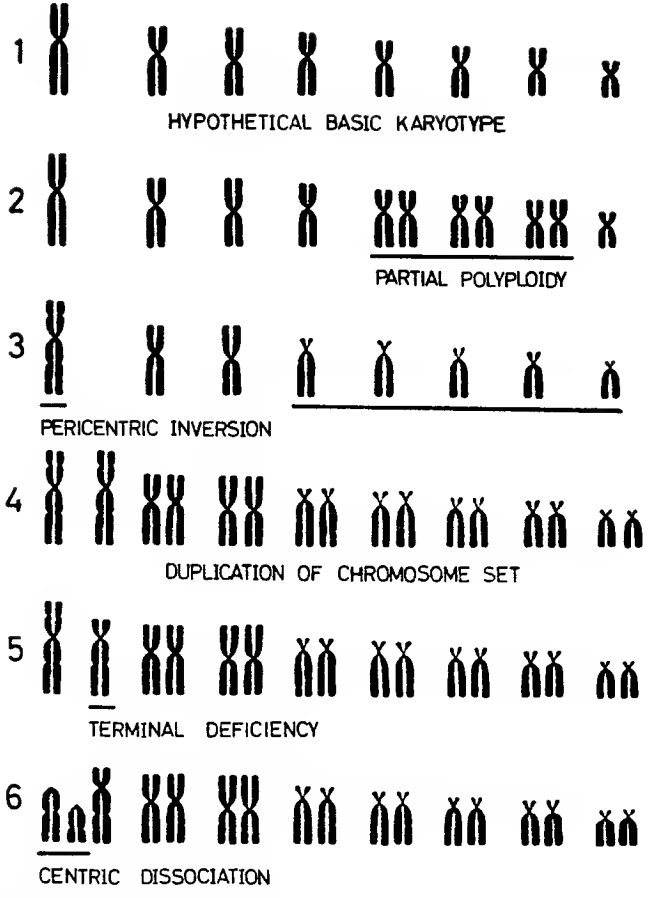


Fig. 3. Hypothetical processes of karyotype differentiation in three Japanese *Aphaenogaster* ants.

1. Hypothetical basic karyotype of *Aphaenogaster*.
2. The karyotype of *A. smythiesi* which can be derived from the hypothetical basic karyotype by partial polyploidy of three submetacentric chromosomes.
3. The first hypothetical step of karyotype evolution which is derived by pericentric inversion from the karyotype shown in 1.
4. The second hypothetical step of karyotype evolution derived by a duplication of chromosome set from the karyotype shown in 3.
5. The karyotype of *A. osimensis* which can be derived by a terminal deficiency of the largest submetacentric chromosome in the hypothetical karyotype shown in 4.
6. The karyotype of *A. famelica* which can be obtained by a centric dissociation of largest submetacentric chromosome of *A. osimensis* shown in 5.

n=1SM+1SM+1M+5SM=8.

Starting from this formula, evolutionary changes in the karyotype of these three species can be hypothesized. The karyotype of *smythiesi* can easily be derived by a partial polyploidy of three medium sized SM chromosomes, possibly through a series of nondisjunctions. Thus the chromosome number increases and the karyotype would be:

n=1SM+1SM+1M+1SM+2(3SM)+1SM=11

The karyotype of *osimensis* might develop through three hypothetical steps. The first step would be the occurrence of pericentric inversions, which produce two types of chromosomes, namely, $1SM^{2SC}$ from the largest SM and 4A from 4SM chromosomes, respectively. The karyotype can be expressed in the following formula:

n=$1SM^{2SC}$+1SM+1M+5A=8.

The second step would be the genome duplication. The chromosome number is doubled to n=16. The chromosome configuration is then:

n=2($1SM^{2SC}$+1SM+1M+5A)=16.

The third step would be a chromosome breakage at the secondary constriction of the short arm of one of the large SM^{2SC} chromosomes. This gives rise to a small sized submetacentric chromosome from the large original one having one secondary constriction on its long arm. The haploid set could be shown by the following configuration:

n=$1SM^{2SC}$+$1ST^{SC}$+2(1SM+1M+5A)=16.

This corresponds to the karyotype formula of the present *osimensis*.

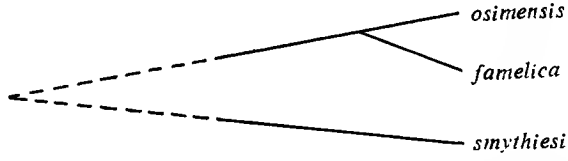
The karyotype of *famelica* could be derived from the karyotype of *osimensis* by centric dissociation of the remaining one large submetacentric chromosome having two secondary constrictions (SM^{2SC}), giving rise to two telocentric chromosomes having one secondary constriction in their long arms. Therefore, the chromosomes would increase to n=17 as shown in the following formula:

n=$2T^{SC}$+$1ST^{SC}$+2(1SM+1M+5A)=17.

These relations are shown schematically in Fig. 3.

The phylogenetic relation of the three Japanese *Aphaenogaster* were morphologically showed o→f→s relation mainly owed to increased rugosity, but the results of both distribution and karyotype comparisons showed another relation o→f // s. So far described, an intimate phylogenetic relation could be concluded between *osimensis* and *famelica* from three lines of evidences, morphological, distributional, and karyotypic, but the phylogenetic position of *smythiesi* deduced from morphology alone does not agree with the other results. As the distribution pattern of *osimensis* conforms to tropical type I and that of *famelica* to temperate type I, then both *osimensis* and *famelica* are considered to belong to "Neo" species. Moreover, based on the karyotype analysis, it was showed that the karyotype of *famelica* was derived from the centric dissociation of the largest submetacentric chromosomes of *osimensis*. On the other hand, the distribution pattern of *smythiesi* conforms to temperate type II, which was found in "Relict" species. The karyotype is characterized by low chromosome number and primitiveness. Although there is a intimate relation on morphology between

famelica and *smythiesi*, still their distribution patterns and karyotypes obviously show a distinct phylogenetic discontinuity between *smythiesi* and *osimensis-famelica* group. Considering from these results, the phylogenetic relation among these three *Aphaenogaster* species can be hypothesized in the following way:



In this connection, Hauschteck (1962) has observed the 2*n* chromosome number of *A. subterranea* to be 22. This number is the same as that of *smythiesi*, which suggests a phylogenetic relation between *subterranea* and *smythiesi*.

The reason for the close resemblance in morphology between *famelica* and *smythiesi* might be due to a convergent adaptation to colder conditions, because many species occuring in high latitudes tend to be covered with a dense pile or rugosae. This morphological marker, increased rugosity, may be useful for identification or comparison among "Neo" species, which are considered as having expanded from the tropical to temperate zone and secondarily adapted to the climate. But if the species to be compared are composed of "Neo" and "Relict" species as in our case, this morphological marker might easily lead to a wrong conclusion, as I have shown above.

The useful morphological character is the leg length and body size of female. It seems interesting that in many ants there is some correlation between body size and chromosome number. Generally speaking, the high chromosome numbered species have large body and rather black color in comparison with related species, for example, *Tetramorium guineense* (n=11)<*T. caespitum* (n=14), *Camponotus* sp. (tropical species) (n=9)<*C. japonica* (n=14), *Pheidole fervida* (n=10)<*P. nodus* (n=19), and *Crematogaster laboriosa* (n=13)<*C.* sp. (tropical type) (n=20). These tendencies are in some agreement with plant evolution in which polyploid plants tend to large size than diploid species (Stebbins, 1950).

Phylogenetic agreement between the results of external morphology and of karyotype analysis have also been established in higher ant such as subfamilies (Brown, 1954; Imai, 1966). However, it has been proved in this study that external morphology by itself is not sufficient in cladistic analysis at the species level.

An effective phylogenetic analysis should include, wherever possible, a combination of morphological, distributional, and karyotypic analysis.

Summary

The chromosome evolution and species differentiation in the three Japanese *Aphaenogaster* ants is studied by means of analysis of external morphology (mainly degree of rugosity), geographic and altitudinal distribution, and karyotype.

The close relation between *osimensis* and *famelica* was established by con-

gruence in the three forms of evidence (morphology, distribution, and karyotype), but the phylogenetic position of *smythiesi* based on morphology alone differed from that based on distribution and karyotype.

The trend in rugosity suggested that *smythiesi* had differentiated from *famelica*. However, the information based on vertical distribution indicates that *smythiesi* is a "Relict" species, that is, an old northern element of the Japanese fauna, while *famelica* was differentiated from *osimensis*, a tropical species that migrated to Japan after the glacial age.

This last conclusion is strongly supported by the karyotype analysis. The karyotype of *famelica* (n=17) could be induced from that of *osimensis* (n=16) by centric dissociation of the largest submetacentric chromosome. On the other hand, *smythiesi* has a primitive karyotype characterized by a low chromosome number (n=11) and many meta- and submetacentric chromosomes. Thus, distributional and karyological considerations suggest a weak phylogenetic discontinuity between *smythiesi* and the *osimensis-famelica* group.

The possible chromosome evolution of these species was discussed, and it was suggested that the karyotype of *smythiesi* has been induced from a common basic number (n=8) by partial polyploidy, while the karyotypes of the other two species were derived by the duplication of chromosome set, pericentric inversion, and centric dissociation, phenomena frequently encountered in the chromosome evolution of ants (Imai, 1969). The limitations of purely morphological evidence in the analysis of phylogeny is demonstrated in this study. It is suggested that the combination of evidence from external morphology, distribution, and karyotype analysis should be used whenever possible in phylogenetic studies of ants.

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Explanation of Plates

Plate 1

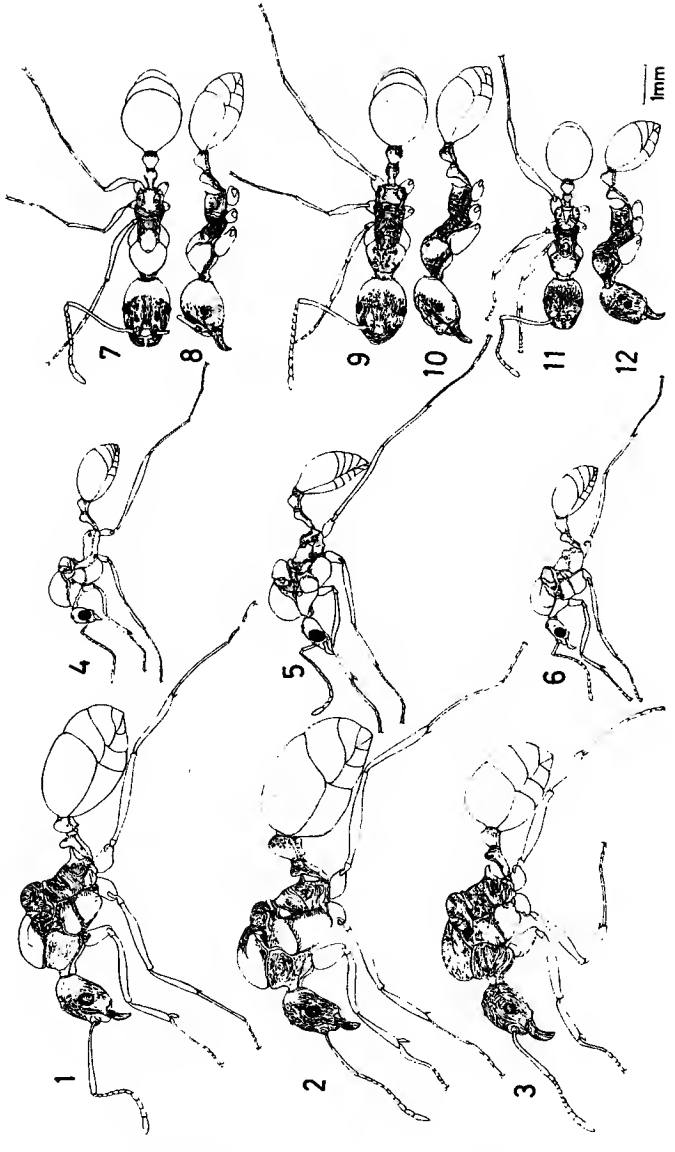
1- 3. Side views of the queens.
1. *A. osimensis*, 2. *A. famelica*, 3. *A. smythiesi*.
4- 6. Side views of the males.
4. *A. osimensis*, 5. *A. famelica*, 6. *A. smythiesi*.
7-12. Side and dorsal views of the workers.
7 and 8. *A. osimensis*, 9 and 10. *A. famelica*, 11 and 12. *A. smythiesi*.

Plate 2

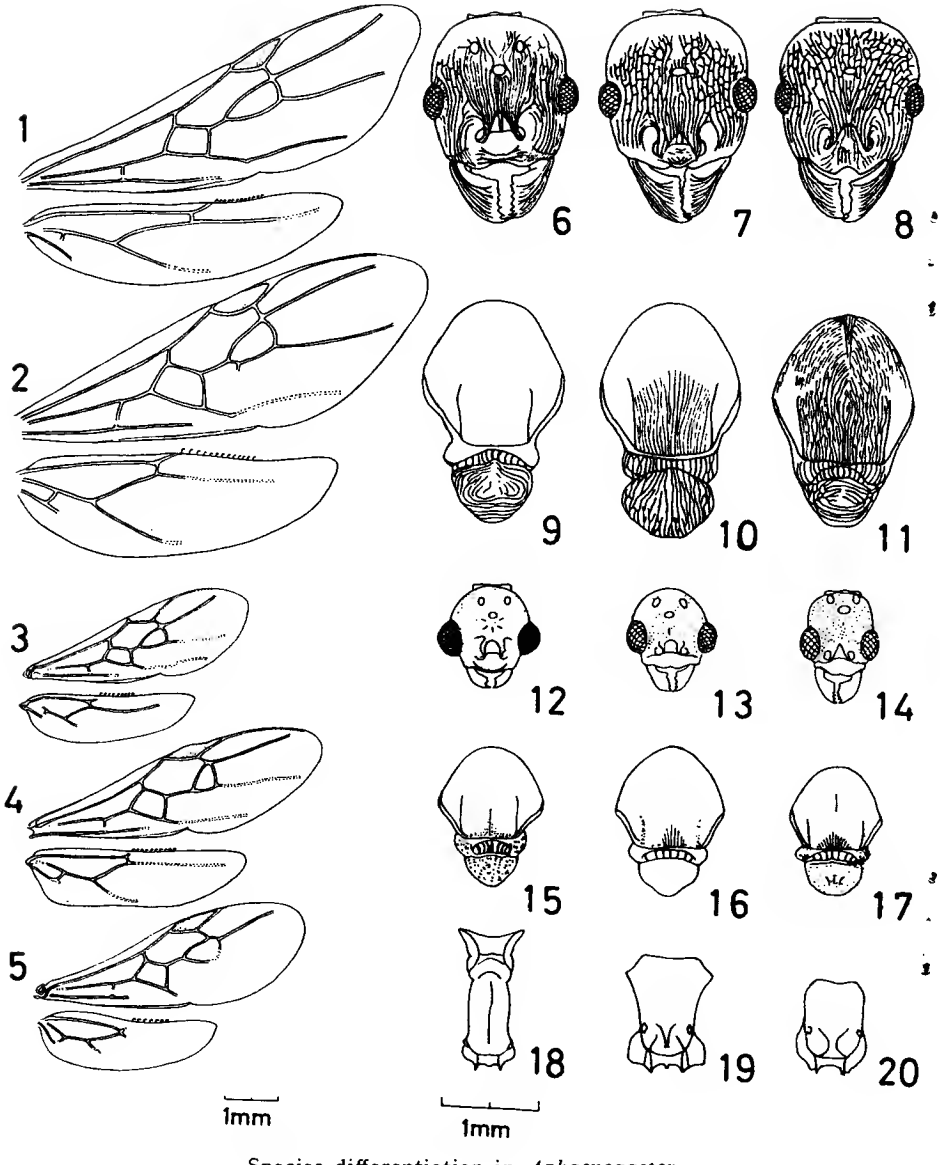
1- 2. Fore and hind wings of the queens.
1. *A. osimensis*, 2. *A. famelica*.
3- 5. Fore and hind wings of the males.
3. *A. osimensis*, 4. *A. famelica*, 5. *A. smythiesi*.
6- 8. Heads of the queens.
6. *A. osimensis*, 7. *A. famelica*, 8. *A. smythiesi*.
9-11. Dorsal views of scutums of mesonotum (upper part) and scutella (lower part) of the queens.
9. *A. osimensis*, 10. *A. famelica*, 11. *A. smythiesi*.
12-14. Heads of the males.
12. *A. osimensis*, 13. *A. famelica*, 14. *A. smythiesi*.
15-17. Dorsal views of scutum (upper part) and scutella (lower part) of the males.
15. *A. osimensis*, 16. *A. famelica*, 17. *A. smythiesi*.
18-20. Dorsal views of epinota of the males.
18. *A. osimensis*, 19. *A. famelica*, 20. *A. smythiesi*.

Plate 3

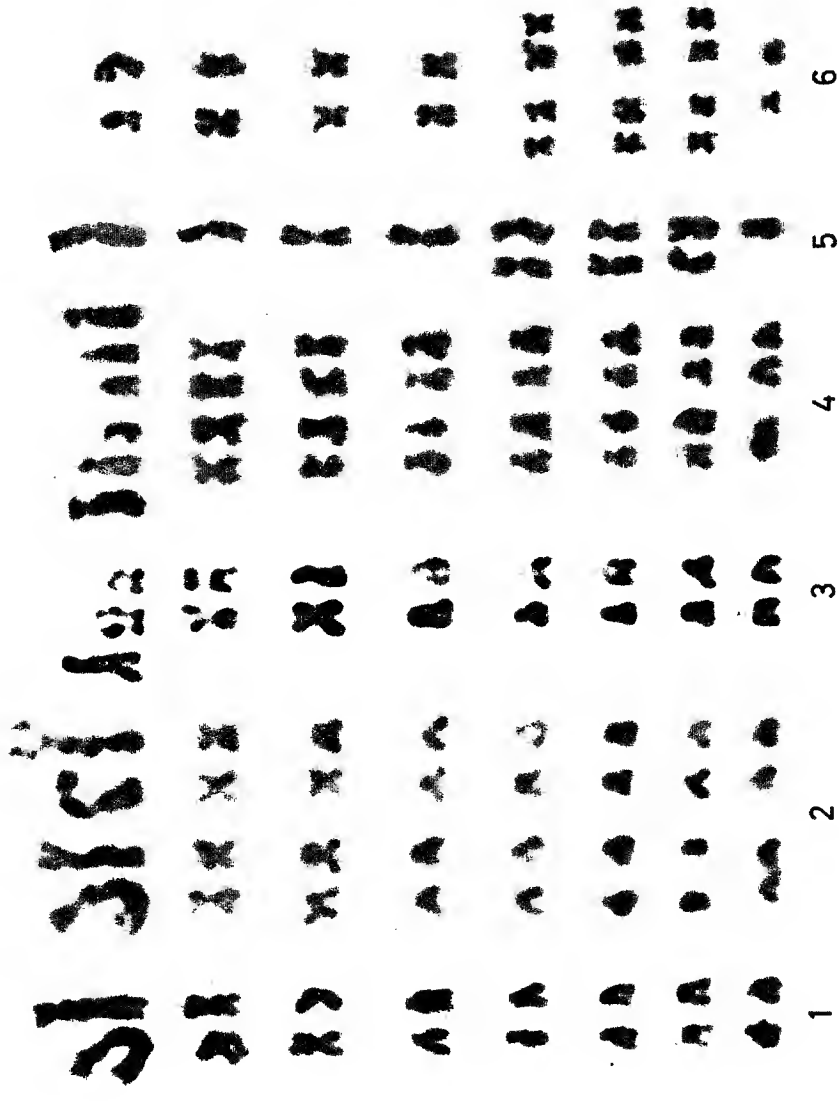
1- 2. Karyotype of *A. osimensis*.
1. Male haploid chromosome set. 2. Female diploid chromosome set.
3- 4. Karyotype of *A. famelica*.
3. Male haploid chromosome set. 4. Female diploid chromosome set.
5- 6. Karyotype of *A. smythiesi*.
5. Male haploid chromosome set. 6. Female diploid chromosome set.



Species differentiation in *Aphaenogaster*



Species differentiation in *Aphaenogaster*



Species differentiation in *Aphaenogaster*